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Robert W. Hutkins
University of Nebraska - Lincoln, rhutkins1@unl.edu

Howard A. Morris
University of Minnesota, St. Paul, Minnesota

Larry L. McKay
University of Minnesota, St. Paul, Minnesota

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Galactokinase Activity in *Streptococcus thermophilus*†

ROBERT HUTKINS,‡ HOWARD A. MORRIS,* AND LARRY L. McKAY
Department of Food Science and Nutrition, University of Minnesota, St. Paul, Minnesota 55108

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ATP-dependent phosphorylation of [^{14}C]galactose by 11 strains of *Streptococcus thermophilus* indicated that these organisms possessed the Leloir enzyme, galactokinase (galK). Activities were 10 times higher in fully induced, galactose-fermenting (Gal$^+$) strains than in galactose-nonfermenting (Gal$^-$) strains. Lactose-grown, Gal$^-$ cells released free galactose into the medium and were unable to utilize residual galactose or to induce galK above basal levels. Gal$^+$ *S. thermophilus* 19258 also released galactose into the medium, but when lactose was depleted growth on galactose commenced, and galK increased from 0.025 to 0.22 μmol of galactose phosphorylated per min per mg of protein. When lactose was added to galactose-grown cells of *S. thermophilus* 19258, galK activity rapidly decreased. These results suggest that galK in Gal$^+$ *S. thermophilus* is subject to an induction-repression mechanism, but that galK cannot be induced in Gal$^-$ strains.

Metabolism of lactose by *Streptococcus thermophilus* is unlike that which occurs in *Streptococcus lactis* and *Streptococcus cremoris*. Whereas both hydrolysis products of lactose, glucose and galactose, are readily fermented by *S. lactis* and *S. cremoris* (6), essentially only the glucose moiety of lactose is utilized by *S. thermophilus*, and galactose is released into the extracellular medium (14, 15). In cultured dairy products made with *S. thermophilus*, galactose accumulates in the milk or curd (11) and may cause product defects (16).

The inability of *S. thermophilus* to utilize galactose may be due to either (i) the absence of one or more catabolic enzymes or (ii) the absence of a galactose transport system. Since the latter possibility would not account for the inability of cells to utilize intracellular galactose, the former explanation is more likely. In addition, galactose transport activity in strains unable to utilize galactose (Gal$^-$) has been reported (5), although transport activity was about 10 times less than in fully induced galactose-fermenting (Gal$^+$) cells and occurred only when an additional energy source, such as sucrose, was also provided.

Recently, Thomas and Crow (14) reported that Gal$^+$ derivatives of four Gal$^-$ parental strains possessed the Leloir pathway enzymes, galactokinase (galK), galactose-1-phosphate uridyltransferase, and uridine diphosphate glucose-4-epimerase. Although the latter two enzymes were also present in the Gal$^-$ strains, galK activity was either very low or undetectable (14).

In this communication, we report that Gal$^-$ strains of *S. thermophilus* phosphorylated galactose via an ATP-dependent enzyme, indicating the presence of galK in these strains. The enzyme was induced by galactose and repressed by lactose.

MATERIALS AND METHODS

Strains and growth conditions. The strains of *S. thermophilus* used in this study are shown in Table 1. All strains were maintained by biweekly transfer in M17 medium (13) containing 14 mM lactose. In addition, Gal$^+$ strains were maintained in Elliker medium (3) containing 27 mM galactose. Some of these strains have been previously described (12).

galK assays. Cells were grown in Elliker broth containing filter-sterilized carbohydrate solutions (27 mM) and harvested after 4 h of incubation at 42°C. Cells were washed and suspended in 20 mM potassium phosphate buffer (pH 7.0) containing 10 mM $MgSO_4$ and 0.5 mM dithiothreitol.

Cell-free extracts were prepared by harvesting 500 ml of midlog-phase cells grown in M17-lactose broth. Cells were washed twice in 20.0 mM phosphate buffer (pH 7.0) containing 10.0 mM $MgSO_4$ and 0.5 mM dithiothreitol and suspended in 3 ml of the buffer. The thick cell suspension was added dropwise into an Eaton press held at −70°C, and a pressure of 10,000 lb/in^2 was used to rupture the cells. DNase (5 μg/ml) was added to the thawed extract and incubated at 15°C for 15 min, after which the debris and whole cells were separated by centrifugation at 10,000 × *g*. The extracts were held at 4°C until used. All assays were performed within 6 h after cells were disrupted.

galK activity was assayed by using the isotope-binding method of Maryanski and Wittenberger (10), with modifications. The assay mixture contained 1.0 mM $MgCl_2$, 1.0 mM dithiothreitol, 100 mM Tris hydrochloride (pH 7.5), 10.0 mM ATP or phosphoenolpyruvate, and 1.0 mM [^{14}C]galactose (0.5 μCi μmol^{-1}; New England Nuclear Corp., Boston, Mass.) in a final volume of 0.19 ml. At 0 time 10 μl of cell extract containing ca. 5 to 20 mg of protein per ml was added and the reaction mixture was incubated at 37°C. Samples (20 μl) were removed with time and spotted on DE81 filter papers (Whatman, Inc., Clifton, N.J.) and air dried. Filters were washed with two 5-ml rinses of twice-distilled water, dried, and counted by liquid scintillation spectroscopy. All counts were corrected for quench. Protein was determined by the method of Lowry et al. (9). galK activity was expressed as micromoles of galactose phosphorylated per minute per milligram of protein. Identification of the galK product was performed by thin-layer chromatography. Portions of the assay mixture were placed in a boiling water bath for 5 min, and debris was removed by centrifugation. Samples were spotted on thin-layer cellulose plates and developed in butanol-acetic acid-water (5:3:2) or 90% ethanol–0.1 M ammonium biborate (6:4). Sections were scraped off the plates into vials, and the radioactivity was counted and compared with known standards.

* Corresponding author.
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‡ Present address: Department of Microbiology, Boston University School of Medicine, Boston, MA 02118.

TABLE 1. Description of *S. thermophilus* strains used in this investigation

Strain	Source[a]	Phenotype[b]
302[c]	INRA	Lac$^+$ Gal$^+$ Glu$^+$
6097	Hansen	Lac$^+$ Gal$^-$ Glu$^+$
SF3	Nestle	Lac$^+$ Gal$^-$ Glu$^+$
3641	NRRL	Lac$^+$ Gal$^-$ Glu$^-$
8	Microlife	Lac$^+$ Gal$^-$ Glu$^+$
YB/ST	Microlife	Lac$^+$ Gal$^-$ Glu$^+$
821	NCDO	Lac$^+$ Gal$^-$ Glu$^+$
573[c]	NCDO	Lac$^+$ Gal$^+$ Glu$^+$
14485	ATCC	Lac$^+$ Gal$^-$ Glu$^+$
19987	ATCC	Lac$^+$ Gal$^+$ Glu$^+$
19258	ATCC	Lac$^+$ Gal$^+$ Glu$^+$

[a] INRA, National Agricultural Research Institute, Jouy-En-Josas, France; Hansen, Chr. Hansen's Laboratory, Inc.; Nestle, Nestle Products Ltd., Lausanne, Switzerland; Microlife, Microlife Technics; NCDO, National Collection of Dairy Organisms, Reading, England; ATCC, American Type Culture Collection.

[b] Lac, Gal, and Glu followed by a + or − superscript indicate the ability or inability, respectively, of the strain to ferment lactose, galactose, or glucose.

[c] These strains are considered Gal$^+$, but grow very slowly in galactose broth.

Sugar assays. Lactose and galactose were assayed using enzyme kits purchased from Boehringer Mannheim Biochemicals, Indianapolis, Ind. (catalog no. 176303).

RESULTS

Many kinase activities can be assayed conveniently by using coupled enzyme procedures in which the oxidation of NADH is measured spectrophotometrically. When the primary enzyme activity is very low or when the background rate of NADH oxidation is very high, these procedures may be inadequate. *S. thermophilus* cell extracts possessed significant NADH oxidase activity, and NADH-coupled assays were difficult to perform, even when the reaction mixture contained excess reducing agents (50 mM dithiothreitol, β-mercaptoethanol, or dithioerythritol). Although galK appeared to be present in Gal$^-$ strains, accurate measurement was not possible with the spectrophometric method (unpublished data).

The isotope-binding method described previously for the measurement of pentitol phosphotransferase activity (8), mannitol phosphotransferase (10), and galK in *S. lactis* (7) is simple, sensitive, and not subject to interference by NADH oxidase or ATPase (if ATP is added in excess). With this method it was possible to detect low levels of galK in all Gal$^-$ strains of *S. thermophilus* examined (Table 2). Galactose phosphorylation occurred only when ATP and not phosphoenolpyruvate was added to the reaction mixture (Fig. 1). Examination of the phosphorylation product by thin-layer chromatography showed that the labeled sugar phosphate cochromatographed with galactose 1-phosphate.

TABLE 2. galK activity in strains of *S. thermophilus*

Strain	galK activity[a] with the following carbohydrate in culture medium:		
	Lactose	Glucose	Galactose
302	0.011	0.003	0.110
6097	0.008	0.017	ND[b]
SF3	0.004	0.004	ND
3641	0.005	ND	ND
8	0.020	0.040	ND
YB/ST	0.028	0.010	ND
821	0.017	0.002	ND
573	0.021	0.046	ND
14485	0.046	0.045	ND
19987	0.011	0.003	ND
19258	0.021	0.020	0.240

[a] Galactokinase activity is expressed as micromoles of galactose phosphorylated per minute per milligram of protein.

[b] ND, Not done.

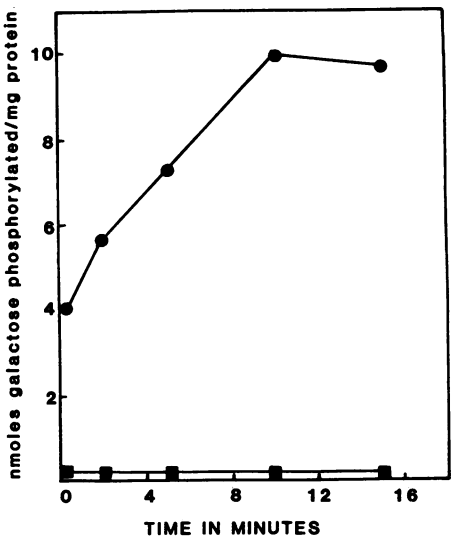

FIG. 1. ATP-dependent phosphorylation of galactose by *S. thermophilus* 19258. A 10-μl sample of a cell-free extract containing 5 mg of protein per ml was added to reaction mixtures containing either ATP (●) or phosphoenolpyruvate (■), and phosphorylation was measured as described in the text.

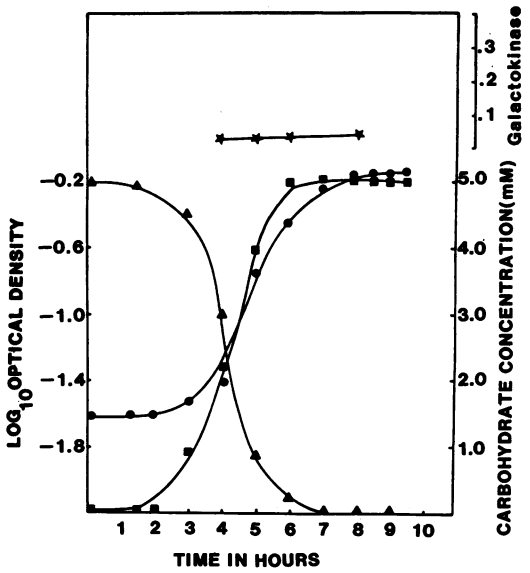

FIG. 2. Utilization of lactose and galactose and induction of galK in a lactose-grown culture of *S. thermophilus* 14485. Cells were grown in Elliker broth containing 5 mM lactose, and galK was assayed at various times. galK activity (★) is expressed as micromoles of galactose phosphorylated per minute per milligram of protein. Other symbols: (●) optical density; (■) galactose; (▲) lactose.

After treatment with alkaline phosphatase, only free galactose could be detected. galK activities were comparable in both glucose- and lactose-grown cultures. However, galactose-grown *S. thermophilus* 19258 had galK activity about 10 times greater than that of the same strain grown in either lactose or glucose, indicating induction by galactose.

When *S. thermophilus* 14485 (Gal^-) was grown in Elliker broth containing 5 mM lactose, only the glucose portion was utilized, and free galactose accumulated in the medium (Fig. 2). Although lactose was eventually depleted, none of the galactose was used. galK activity was low and remained constant throughout growth (ca. 0.045 μmol of galactose phosphorylated per min per mg of protein). *S. thermophilus* 19258 (Gal^+) grown in 5 mM lactose medium also initially utilized only the glucose portion and released free galactose (Fig. 3). However, when lactose had been depleted galactose utilization subsequently occurred until after 10 h, when all available carbohydrate had been fermented. galK activity was initially low, as with strain 14485, but rose from 0.025 to 0.22 μmol of galactose phosphorylated per min per mg of protein, about a 10-fold increase in activity.

When *S. thermophilus* 19258 was grown in 20 mM galactose medium, galK activity was initially very high (Fig. 4). However, the addition of 5 mM lactose caused a rapid reduction of galK to levels approaching that of the lactose-grown cultures during the early stages of growth. Lactose was preferentially utilized, and release of galactose occurred.

DISCUSSION

Although most lactic acid bacteria which ferment lactose utilize both monosaccharide components, there are also reports which indicate that some microorganisms utilize only one of the two sugars. *S. lactis* C10 released free galactose into the medium when grown in lactose broth (4), although to a much lesser extent than does *S. thermophilus*. Lactose-grown *Escherichia coli* mutants which are lactose positive (Lac^+) and glucose negative (Glu^-) release glucose and galactose into the extracellular medium and then utilize the galactose moiety, but not the glucose moiety (1). When grown on lactose, a strain of *Arthrobacter globiformis* releases galactose, but utilizes the glucose (2). The fermentation pattern obtained when *S. thermophilus* 19258 is grown on lactose is unique in that this strain is phenotypically Gal^+. However, utilization of galactose was expressed only when lactose was absent and galactose was present in the medium. *S. thermophilus* 14485 was unable to utilize any of the available galactose generated by lactose hydrolysis even when lactose had been depleted and the pH was maintained at 6.0 with 75 mM phosphate buffer. Other strains, however, utilized a small amount of galactose while lactose was still present in the medium (data not shown). Similar results were also obtained by Tinson et al. (15), and recently Thomas and Crow (14) showed that Gal^- strains TS2, MC, and 821 released less galactose than the total available from lactose hydrolysis, indicating partial utilization of this sugar.

The recent report by Thomas and Crow (14) that Gal^+ strains of *S. thermophilus* possessed enzymes of the Leloir pathway was supported by our findings of galK activity in our strains. The elevated activities of galK observed in Gal^+ *S. thermophilus* 19258, as compared with Gal^- strains, is consistent with the activities reported by Thomas and Crow (14). That galK activity in strain 19258 was higher when cells were grown in galactose than in glucose indicated that galK was induced by galactose. Although diauxic growth on lactose was not observed with strain 19258 or in strain TS2b, as reported by Thomas and Crow (14), it was evident that a

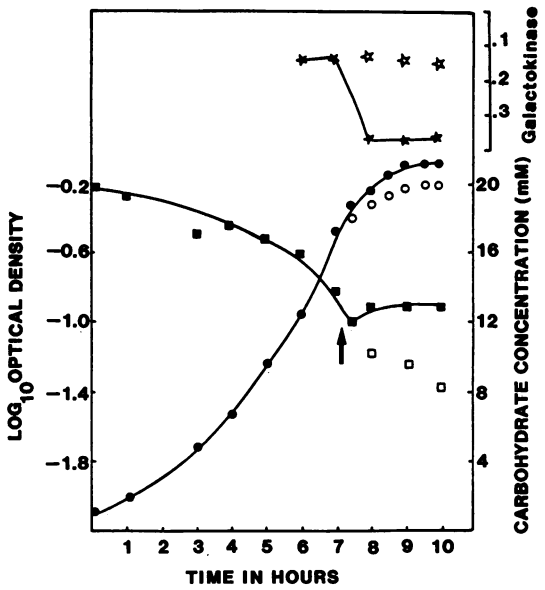

FIG. 4. Utilization of galactose and effect of lactose on galK activity in *S. thermophilus* 19258. Cells were grown on Elliker broth containing 20 mM galactose, and galK was assayed at various times. After 7 h (as indicated by the arrow) the culture was split, and lactose (5 mM) was added to a portion. Symbols: (★) galK in experimental culture; (☆) galK in control (no lactose added); (■) galactose in experimental culture; (□) galactose in control; (●) optical density in experimental culture; (○) optical density in control. galK activity is expressed as micromoles of galactose phosphorylated per minute per milligram of protein.

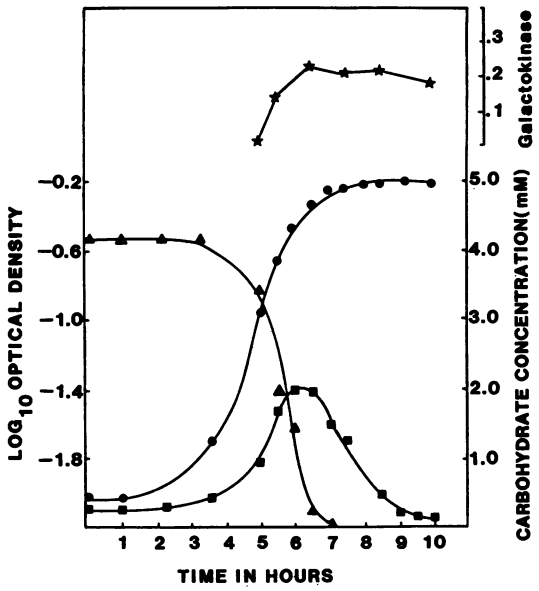

FIG. 3. Utilization of lactose and galactose and induction of galK in a lactose-grown culture of *S. thermophilus* 19258. Cells were grown in Elliker broth containing 5 mM lactose, and galK was assayed at various times. galK activity (★) is expressed as micromoles of galactose phosphorylated per minute per milligram of protein. Other symbols: (●) optical density; (■) galactose; (▲) lactose.

switch from glucose utilization (as lactose) to galactose utilization had occurred when lactose was depleted. In addition, recent evidence (5) indicated that galactose transport in strain TS2b is induced by galactose. However, the immediate reduction of galK in *S. thermophilus* 19258 when lactose was added to galactose-grown cells (Fig. 4) and the absence of appreciable galK activity in 4-hour lactose-grown cells (when galactose was also present) (Table 2) indicate that galK may also be subject to catabolite repression by lactose. Since galK and galactose transport activities (data not shown) were also low in glucose-grown strain 19258, glucose generated by lactose hydrolysis may also act as a repressor. The inability of lactose-grown, Gal^- strains to induce galK when lactose had been depleted, in contrast to the Gal^+ strain 19258, which could, might be due to a permanently repressed gene coding for galK in the former strains. Although most strains in our collection are Gal^- and have low galK activity, the report of Thomas and Crow (14) suggests that other Gal^+ strains of *S. thermophilus* can be obtained from Gal^- stock cultures.

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